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(54) Title: NEW USE OF INDOCYANINE GREEN AS A PHOTOSENSITVE AGENT

(57) Abstract: The present invention is directed to a new use of indocyanine green as a photosensitive agent in a dinamic photothermotherapy and selective dynamic thermotherapy for occlusion of choroidal neovascularization.

NEW USE OF INDOCYANINE GREEN AS A PHOTOSENSITIVE AGENT.

"NEW USE OF INDOCYANINE GREEN AS A PHOTOSENSITIVE AGENT IN DYNAMIC PHOTOTHERMOTHERAPY AND SELECTIVE DYNAMIC THERMOTHERAPY FOR OCCLUSION OF CHOROIDAL NEOVASCULARIZATION; NEW USE OF A LASER THERAPY SYSTEM AND DELIVERY OF WAVELENGTH LIGHT IN THE INFRARED SPECTRUM FOR ACHIEVING PHOTOTHERMO DYNAMIC EFFECT, AND SELECTIVE VASCULAR OCCLUSION OF THE CHOROIDAL AND/OR THE RETINA OR THE SUBRETINAL SPACE, ASSOCIATED WITH SAID INDOCYANINE GREEN, AND DYNAMIC PHOTOTHERMOTHERAPY AND SELECTIVE DYNAMIC THERMOTHERAPY PROCESS FOR SUBRETINAL VASCULAR OCCLUSION USING INFRARED LASER DELIVERY SYSTEM AND INDOCYANINE GREEN"

Field of Invention

The present invention relates to a new use of indocyanine green as a photosensitive agent in dynamic photothermotherapy and Selective Dynamic Thermotherapy for occlusion of choroidal neovascularization, in patients with age-related macular degeneration, pathological myopia, angiod strias, syndrome of presumed ocular histoplasmosis, inflammatory or idiopathic causes, henceforth named *i*-PTT and *i*-TTD; also refers to a new use of a Laser Therapy System and Delivery of Wavelength Light in the Infrared Spectrum for achieving Photodynamic Effect, and Selective Vascular Choroidal Occlusion using indocyanine green in its original formula; as well as, in modified presentations with Liposome Encapsulation, with the objective of increasing its half-life, reducing aggregation and larger intra-muscular selectivity, as well as other chemical modifications or the synthesis route, and refers to a photothermodynamic therapy and Selective Dynamic Thermotherapy process for subretinal vascular occlusion, using said infrared laser delivery system and indocyanine green.

Background of the Invention

Indocyanine green, an anionic tricarbocyanine dye has been used for imaging retina and choroidal vasculature for over 30 years. There are several scientific works showing improvements in this technique or in imaging by digital video-angiographies, and that detach several computer programs with the objective of working the image achieved by this recording method.

This relatively large photosensitive molecule, binds almost totally to plasmatic proteins originating a protein-indocyanine green complex. (Paumgartner G, Probst P, Kraines R, Leevy C. Kinetics of indocyanine green removal from the blood. *NY Acad Sci* 1970; 170:134-170), and presenting an absorption peak of about 805 nm, near the emission peak at 810 nm of the conventional diode laser. Consequently, its use in relation to other dyes constitutes an additional advantage because of deeper tissue penetration of this wavelength laser able to excite the intra-tissue dye in choroidal vessels and subretinal tissue (Anderson R, Hu J, and Parrish J. Optical radiation transfer into human skin. In: Marks R, Payne P, editors. Bioengineering and the Skin. Boston: Lancaster MTP, 1981:253-265, Grossweiner LI. The Science of Phototherapy. Boca Raton, Fla.: CRC Press, 1994; 27-49,139-155,175-177 London).

Additionally, indocyanine green dye, causing minimal damages to under laying tissue, like other second-generation agents used in photodynamic therapy, is characterized by low skin phototoxicity, high tissue targetability, rapid biodistribution and clearance, as well as technically easy administration and monitoring, indispensable qualities for an ideal photosensitizer to be applied in photodynamic therapy. The use of indocyanine green in combination with infrared

light has been appointed as photosensitizing agent for photodynamic therapy of colonic cancer cells. (Baumler W, Abels C, Karrer S, *et al*. Photo-oxidative killing of human colonic cancer cells using indocyanine green and infrared light. *Br J Cancer* 1999;80:360-363).

To the best of our knowledge, the potential of photothermodynamic therapy mediated by indocyanine green in the choroidal vessels and choriocapillaris and/or choroidal neovascularization, subretinal space and/or retina have never been reported in the literature.

Photodynamic therapy leads to cell death due to the generation of singlet type oxygen and the subsequent formation of oxidative substances as lipid peroxides, post-photoactivation by a wavelength diode laser at 805 nm. Photodynamic therapy can be applied by a therapeutic modality that involves the excitation of a dye by light of a specific wavelength, with the release of excited oxygen and consequent activation of clotting reaction in cascade.

Due to recent limitations referring to the approval by FDA involving this type of therapy for subretinal neovascular membranes, predominantly classics in the subfoveal region, correlated treatments are presently under evaluation in an effort by all skilled scientific community, aiming to the improving of final results involving this disease, clinically destructive for the vision, which affects over 200 thousand patients, per year, in the United States, which is the leading cause of blindness, from a legal point of view in patients over 60 years).

Age-related Macular Degeneration is the leading cause of legal blindness in people over 60 years in the developed countries.

Presently, bearers of choroidal neovascularization in the subfoveal region have several options for their treatment; however, there are various limitations regarding the results achieved.

Laser photocoagulation of subretinal vascular membrane in Exudative Macular Degeneration is a well-studied procedure, this therapeutic modality is the only treatment accepted, until recently, but usually with negative results.

Its indication is limited to a small quantity (minority) of patients wherefrom, can be observed by fluorescein angiography, a classic and/or well-defined neovascularization which limits are well-outlined, and thereby, with the identification of all extension of the neovascular complex, perform photocoagulation by laser, aiming at destroying the subretinal neovascular lesion, that, however, causes the destruction of the neurosensory retina, creating as a consequence, an escotome with a subdivision related with said treatment, which, on the other hand, has a better visual acuity over time, after one or two years, in relation to the natural history of the disease.

Although this treatment is enough destructive, it is associated with a large and non-acceptable persistency and/or recurrence of neovascularization, over fifty (50%) percent of the cases.

Another type of treatment that was approved by FDA for subretinal neovascularization, has been the application of photodynamic therapy using verteporfin (Visudyne) that, however, also presents significant limitations since, up to now, it was released only for treating the membranes predominantly or exclusively classics.

Only forty-percent (40%) of the patients with Exudative Macular Degeneration, within this criteria, have great possibility of benefiting from the treatment.

Another disadvantage is related with the high cost of photosensitizing agent, mainly because the need of frequent applications occurs invariably at an average of 3.4 in the first year and 2.1 in the second year that constitutes important and limiting aspects of the indications and maintenance of this treatment over time. (Miller JW, Schmidt-Erfurth U, Sickenberg M, *et al.* Photodynamic therapy with verteporfin for choroidal neovascularization caused by age-related macular degeneration. Results of a single treatment in a phase 1 and 2 study. *Arch Ophthalmol* 1999; 117:1161-1173 and Schmidt-Erfurth U, Miller JW, Sickenberg M, *et al.* Photodynamic therapy with verteporfin for choroidal neovascularization caused by age-related macular degeneration. Results of retreatments in a phase 1 and 2 study. *Arch Ophthalmol* 1999; 117:1177-1187 and TAP Study Group. Photodynamic therapy of subfoveal choroidal neovascularization in age-related macular degeneration with verteporfin. One-year results 2 randomized trials-Report 1. *Arch Ophthalmol* 1999; 117:1329-(1345).

Therefore, it has been trying to develop new modalities for treating subretinal neovascular membrane, which may then be grouped in four major categories. These therapies are divided as follows:

a) photodynamic therapy with new photosensitizers;

b) pharmacological agents able to inhibit or control the formation of neovascularization with anti-angiogenic agents;

c) surgical treatment, including limited and/or extensive macular translocation with retinothomy at 360 degrees and also with or without exeresis of subretinal neovascularization;

d) radiotherapy.

The first modality of treatment with laser photocoagulation had the function of burning the damaged region; such technique, therefore, showed the great disadvantage of leaving a turbidity or permanent marker in the central visual field of the patient, besides definitely damaging the neurosensory retina of the region treated.

After this photodynamic therapy process by applying laser photocoagulation was also used photocoagulation with laser at 810 nm, associated with the use of indocyanine green to induce the thermal occlusion and photocoagulation of choroidal neovascularization in combination with the fact of having greater light absorption by the dye, inducing the increase of thermal photocoagulation effect itself. However, such process showed no significance and/or particularity in terms of positive results of the treatment, since its effect was mainly thermal photocoagulating, leading to an undesirable large tissue lesion in tissues adjacent to said proper neovascular membrane.

There from, another method related with the use of a photosensitization without significant heat reaction, associating a medicine developed by QLT/CIBA Vision to photodynamic therapy was developed, thereby, creating another treatment modality under development in the medical field, more specifically in ophthalmologic sector, aiming, by this technique, at the selective occlusion of choroidal neovascularization by means of a non-thermal determined

reaction and, thereby, sparing the primary structures for recovery and/or improvement of recovery of visual acuity.

However, only 20 to 40 percent of patients with Age-Related Macular Degeneration, exudative type, present a potential to benefit of the treatment. Specific indication for the patient to present the conditions to undergo the treatment, are the classic membranes lower than 6,4 mm in its larger linear extension since, the treatment and the need of at least 3.4 applications in the first year and 2.1 in the second year, in average, are important limitations for this type of treatment.

Several efforts have been applied by those studying this issue, in order to prepare or test new photosensitizers for photodynamic therapy, which can be effectively applied, at lower costs, in a larger group of patients with Age-Related Macular Degeneration, aiming at better improvement of the visual result for a larger number of patients bearing this disease.

Laser application at 810 nm, in treating subretinal neovascular membrane, with beams in the retina smaller than 1.000 micros, whether associated or not with the use of Indocyanine Green, produces heat generation and, as a consequence, the eye treated may have its neurosensory retina definitely damaged due to the intensity used for the treatment.

Another attempt was made in order to use the diode laser tuned to 810 nm, with beams higher than 1.000 micros, aiming at treating the lesion by means of heat generation using retinal irradiation inferior to that needed for photocoagulation (hyperthermy/thermotherapy). The rationality of the therapy proposed is highly questionable, since lack the selectivity needed for treating the disease at issue;

and, as suggested in the name of said treatment, has the effect based on hyperthermy generation or heat generation and risk of developing tissue burns with loss of tissue particularity in a treatment that spares the neurosensory retina and, thereby reducing the possibility of maintaining and/or improving the visual acuity over time.

Patent US 6.140.314 at "The John's Hopkins University" ownership, published on 12/31/2000, describes methods and material for visualization or general treatment of the vasculature or blood vessels in the eyes, with a method that involves intravenous co-administration of a fluorescent dye encapsulated with liposome, responsive to heat or heating, and an individual agent, rational and efficient to cause a chemical tissue damage after its non-invasive activation in a pre-determined anatomic area in the eye, in such a way that the liposomes responsive to heat, leak and deliver its content inside the blood vessels in the pre-determined local, so that the fluorescent dye can visually be noted forming a mark in the vasculature, which then, fluoresces activated by a mechanism sufficient to occlude the vessels, thereby, producing an occlusion in the area activated by the laser; this process can be repeated provided that liposome are systematically circulating after its administration. In this reference, however, it is not specifically evidenced the use of Indocyanine Green as a photosensitizing agent activated by a light excitation of "low power laser", insufficient to generate heat or photocoagulating hyperthermy and/or able to induce tissue burn, as defined by this invention.

Summary of Invention

This invention has the purpose of using a laser system modified under pressure of the effect of photodynamic therapy for vascular occlusion of choroidal vessels or neovascularization originating from choroids that invade the subretinal tissue or neurosensory tissue itself, in a selective form, achieving the intravascular coagulation by photothermo-dynamic effect without significantly damaging and/or by heating tissues and/or by hyperthermy of adjacent tissues, that is, the treatment spot until the outgoing of new use of the dye and photosensitizer indocyanine green as an agent in the photodynamic therapy to be used in the diseases: age-related macular degeneration, pathological myopia, angiod strias, syndrome of presumed ocular histoplasmosis, inflammatory or idiopathic causes and other abnormalities that may generate enlargement of abnormal vessels in ocular tissues.

The Dynamic Photothermotherapy and the Selective Dynamic Thermotherapy process proposed uses a photochemical interaction and photophysics, infrared wavelength light emitted by a diode laser, exposure area of up to 8.6 mm diameter, and using only one round and/or modified beam, customized in accordance with the targeted tissue.

Therefore, the present invention is centered on the following purposes:

1) The Dynamic Photothermotherapy process in this invention is directed to the use in abnormal vessels of ocular tissues, particularly in choroids, retina and/or between the choroids and the retina, which are leaking fluid and/or blood to the referred tissues, and thereby, increasing the risk and/or causing visual decay.

2) The process uses infrared laser in wavelength band, which has more affinity or absorption with the photosensitizing dye used.

3) Use of a dye in its natural form and/or modified by liposome encapsulation and other chemical alterations, such as lyophylization, inducing the diminishing of aggregation or intravascular selectivity in a great photophysics and photochemical reaction affinity, which has great interaction with regular indocyanine green and/or modified by liposome encapsulation and other chemical alterations.

4) Use of delivery systems already improved at laser light, for light excitation without causing thermal tissue reaction, typical of photocoagulation, causing only, photodynamic effect and/or hyperthermy that generates temperature increase, insufficient for clotting or burning adjacent tissues, and that is not a spot of treatment selection.

5) Use of ambulatory and/or surgical treatment process that do not require anesthesia by means of tissue injection, but only in its topical form. Therefore, constitutes a process where a projection of the laser light to be projected, can be used, while there is no said laser shot, with no photothermodynamic effect occurring unless, at the moment when light excitation is activated to induce the reaction with the photosensitizing agent. Activation of the photosensitizing agent indocyanine green occurs *in vivo*, after light exposure at a dose, intensity, luminance and power, in accordance with the best selective occlusion effect.

The procedures are based on administration of a photosensitizing agent in intravenous form, waiting until the dye has reached a proper maximum distribution and localization inside the pre-selected area for treatment, the injection can be repeated according to the case and/or cause

of the vascular alteration to be treated. Next, a medical instrument is used associated with an infrared laser light source, and thereby inducing the interaction between the light and the photosensitizing agent, during specific time determined. Excitation of the photosensitizing agent concentrated in the targeted tissue, during the predetermined time while observing the tissues being treated and eventually any visible direct or indirect effect by means of an optical instrument coupled to the emitting source of laser light.

6) Use of a contact or non-contact lens for focalization of the source of infrared laser on the targeted tissue to be treated. Administration of photosensitizing substance with light excitation for proper therapeutic effect.

7) System of specific focalization of the laser light for larger concentration of the photosensitive dye on the targeted tissue, for achieving the tissue reaction induced by photodynamic reaction.

Therefore, the present invention constitutes in the use associated of the photosensitizing agent that will be exposed to the brightness. by the projection of the light of the infrared laser in wavelength strip capable to promote the absorption of the used dye, in that the heat generated by the luminous stimulation it happens in controlled thermal conditions, and with great photo-physic and photochemical interactions, so that it doesn't cause characteristic thermal photocoagulation reaction, but just causing photodynamic effect and/or insufficient hyperthermy to cause damages to the adjacent tissues.

Additionally, it is an objective of the present invention, the uses of the photosensitizing agent in pre-defined conditions, the Indocyanine Green, associated to the exposure to the luminous agent, the light of the infrared

laser, to reach the Dynamic Photothermotherapy (i-PTT) and the Selectively Dynamic Thermotherapy (i-TTD) for occlusion of choroidal neovascularization. The photosensitizing agent will be exposed to the brightness by the projection of the light of the infrared laser in wavelength strip capable to promote the absorption of the used dye, in that the heat generated by the luminous stimulation it happens in controlled thermal conditions, and with great photophysics and photochemical interactions, so that it doesn't cause characteristic thermal photocoagulation reaction, but just causing photodynamic effect and/or insufficient hyperthermy to cause damages to the adjacent tissues.

In the same way, with the control and decrease of the thermal component in the mechanism of action, it is also possible to obtain a variant of the process with smaller photodynamic effect, however being still present the use of the 2 (two) components, constituting the mechanism of the Selectively Dynamic Thermotherapy (i-TTD).

The methodology for the development of the present invention had the scientific discovery as the basis that Photodynamic Therapy is a system of treatment with relatively recent development, when it was evidenced that phototoxic responses to photosensitizing agents could be achieved by means of experiments performed in 1900 (*Raab O. Uber die wirkung fluoreszierenden stoffen. Infusuria Zeitschrift Biologic 1900; 524-546*). Scientific Articles in 1970 decade describing the successful treatment of tumors in animals using hematoporphyrin administered intravenously have developed the modern era of photodynamic therapy (*Dougherty TJ, Grindey GB, Fiel R, Weishaupt KR, Boyle DG. Photoradiation therapy II: cure of animal's tumors with*

hematoporphyrin and light. J Nat Cancer Inst 1975; 55:115-121).

By this process is administered an intravenous injection of a photosensitizer agent, which accumulates in the neovascular tissue and in tumors or tumor tissues. The photosensitized tissue with the administered substance is then irradiated by a light that has its maximum absorption peak equated to an interactive effect carrying the phototoxicity induced by the equation: tissue-photo sensitizer-light, specific selective (*Manyac MJ, Russo A, Smith PD, Glatstein E. Photodynamic therapy. J Clin Oncol 1988;6:380-391 and Gomer C. Photodynamic Therapy in the treatment of malignancies. Semin Hematol 1989; 26:27-31*).

The result of randomized scientific studies, double blind (masked) and multicentric, benzoporphyrin derivative monoacid photodynamic agent (BDP verteporfin, Visudyne; CIBA Vision, Duluth, USA), has recently received the United States Food and Drug Administration approval for clinical use in Age-Related Macular Degeneration and with subretinal neovascular membranes, predominantly classics (Miller JW, Schmidt-Erfurth U, Sickenberg M, *et al*.Photodynamic therapy with verteporfin for choroidal neovascularization caused by age-related macular degeneration. Results of a single treatment in a phase 1 and 2 study. *Arch Ophthalmol* 1999;117:1161-1173 and Schmidt-Erfurth U, Miller JW, Sickenberg M, *et al*. Photodynamic therapy with verteporfin for choroidal neovascularization caused by age-related macular degeneration. Results of retreatments in a phase 1 and 2 study. *Arch Ophthalmol* 1999; 117:1177-1187 and TAP Study Group. Photodynamic therapy of subfoveal choroidal neovascularization in age-related macular degeneration with

verteporfin. One-year results 2 randomized trials-Report 1. *Arch Ophthalmol* 1999; 117:1329-(1345).

Unfortunately, about 60% of the patients with subfoveal choroidal neovascularization related with Age-Related Macular Degeneration, are not eligible for treatment due to angiographic characteristics, since they show occult and/or minimally classic neovascular membranes (Marguerio RR, Marguerio AR, DeSantis ME. Laser treatments with verteporfin therapy and its potential impact on retinal practices. *Retina* 2000:20;325-330).

The Searchers have been seeking to identify new photosensitizers for photodynamic therapy that can be efficiently used in larger groups of patients bearers of Age-Related Macular Degeneration and that are able to improve the visual acuity in this disease and in others that have the development of choroidal neovascularization and/or subretinal and/or retinal.

Based on this discovery, this invention consists of the determination of conditions under proceeding phases to achieve an evaluation of the vascular tissues response, in particular, choriocapillaris and choroidal neovascularization, in relation to the indocyanine green, in photophysics and photochemistry (photodynamic) in relation/interaction of indocyanine green and of the light in 810 nm length. Studies were carried out in pigmented and non-pigmented rabbits eyes.

Large and deeper choroidal vessels and other extra ocular components, as neurosensory retina and retina pigment epithelial, were evaluated in relation to eventual damages and tissue lesions arising or followed by photodynamic therapy with indocyanine green.

For performing the treatment involved in the context of the present invention, conventional angiography tests with sodium fluorescein and Indocyanine Green are used, since the angiographic method directed by laser and occlusion is, therefore, totally different from conventional fluorescein angiography and/or Indocyanine angiography used, appointed herein.

However, within the context of this invention, Indocyanine Green used here as photosensitizer can be modified by several means for treatment by dynamic photothermotherapy, can as well be associated with other forms of photocoagulating hyperthermy, that is, being unable to induce tissue burns, also becoming selective, thereby.

Aiming at equating the problems existing in this issue technique, this invention is proposing a new photothermo-sensitizing process, which involves an intravenous injection of Indocyanine Green at doses from 0,5 to 5,0 mg/Kg, preferably, 2 mg/Kg and focal activation with laser light of 810 nm in a retinal irradiance, where the energy absorb by the molecule is not primarily converted to heat (photo-oxidation type I), but transferred to molecular oxygen via triplet state (photo-oxidation reaction type II).

This association of the wavelength laser of 700 to 900 nm (preferably 810 nm), at low power inferior to 1.000 miliwatts and extended exposure time from 40 and 150 seconds (preferably 100 seconds), for areas of circular application of minimum 1.600 micros, generating retinal irradiances, thereby, never higher than 4,0 WATTS/square centimeters, conferring a satisfactory response, primarily by means of photo-oxidation effects type II, with acuity stabilization or improvement; in general, with improvement of relative escotome, with no collateral damages and with low rates of

retreatment of the subretinal neovascular membrane, in addition to the extremely low cost of the medication proposed herein (Indocyanine Green) and its application for almost the totality of subretinal neovascular membrane cases.

Authors of the present invention, as from the evaluation of choriocapillaris response (choroidal vascular tissue topographically situated in choroidal inner layer, near Bruch's membrane, with wavelength light at 810 nm and Indocyanine Green in pigmented and non-pigmented rabbits eyes, achieved positive results in order to evaluate the medicinal and technological potential of the present invention and dynamic photothermotherapy, for choroidal neovascularization occlusion, vascular tissue bellow the retina, which includes choriocapillaris and external deep vessels, where it originates the neovascularization lately noted in humans).

In the examination at issue, authors have used background (fundus) photography, fluorescein angiography, and light and transmission electron microscopy to study the efficiency of photodynamic therapy induced by photothrombosis using Indocyanine Green as the photosensitizer agent, excited by the wavelength light at 810 nm, measured by a wavelength diode laser that would be near the maximum absorption peak of Indocyanine Green, as a modified and improved delivery system manufactured by Opto.

The choriocapillaris occlusion was achieved with Indocyanine at doses of 10 to 20 mg/Kg and irradiation as low as 7,2 Joules/cm^2.

When the dynamic photothermotherapy was performed with an Indocyanine Green at doses of 10 mg/kg, the damage found in the neurosensory retina was absent or minimal. Inner photoreceptor segments showed degeneration, probably

secondary to choroidal ischemia. Brüch's membrane remained intact, pigmented epithelium was invariably damaged by the treatment, however, in the same manner noted with other photosensitizers previously tested, even those of second-generation.

Temporary occlusions of large choroidal vessels occurred at both doses, over and bellow 10 mg/Kg.

The present invention reports for the first time a case of occult type choroidal neovascularization due to Related Macular Degeneration that was successfully treated by the dynamic photothermotherapy with Indocyanine Green (*i*-PTT).

Since this experiment was successfully performed showing viability of the photothermo-dynamic effect, and since no burn mark was noted in the neurosensory retina, either from a clinical point of view, or from a histological point of view, with optic and electron microscopy, went through investigation, except on the reaction induced by the process at issue.

Indocyanine Green is a water-soluble tricarbocyanine molecule that does not contain more than 5% sodium chloride, chemically is N-hydro-3,3', 3'-tetrametyl-1,1'-di-(---- sulphobutyl)-4,5,4',5'-hidroxy of benzoindotricarbocyanine with molecular weight of 775.

Its empiric formula is $C_{43}H_{47}N_2NAO_6S_2$.

In addition to its high molecular weight, Indocyanine Green shows high bound level to plasma lipoproteins (approximately 98%, warranting intravascular retention properties).

Fluorescence properties assure the exact contrast localization in subretinal neovascular lesion, prior to positioning the laser beam for application of activating light.

Thus, after administration of indocyanine green during the semiologic process, and after observing the concentration of this substances in the neovascular membrane itself, we are certain of the bio-assessibility of the medication to achieve the desired effect of photothermodynamic and neovascularization selective occlusion of which the substance is concentrated.

Taking the concentration effect during the propedeutic investigation and/or analysis process as basis, we found that it is possible to have certainty of the substance localization in the neovascular membrane, within the performance of therapeutic procedure, and for this reason, after this previous clinical evidence, the photodynamic therapy, thereby, having certainty that after administration of the photosensitizer dye, the same will be concentrated in the lesion itself, and its effect will occur in a selective manner.

Indocyanine Green constitute a medicament relatively cheap and safety that has a very low local and systemic toxicity and a favorable biodistribution, as well as fluorescence properties already known, which detection technology was largely improved in late years through reaching digital images and video angiography cameras able to actuate in infrared band.

Are commercially available, and distributed in a generic and ample form, in all ophthalmologic market. The medication is easily administered, and is rapidly distributed and excreted. Up to now, Indocyanine Green was not shown for photodynamic therapy treatment, until the authors of the present invention could show *in vivo* (*Costa et al*, unpublished data, 2000).

Additionally, a modified wavelength diode laser at 810 nm, similar to the one used for photodynamic therapy with Indocyanine Green, was used with safety to deliver irradiances of about 7.000 milliwatts/cm² in 60 seconds for treatment of occult subfoveal subretinal neovascularization. Activation of about 805 nm is also advantageous due to the deep large light penetration. If all these factors are considered, these preliminary studies determine the safety criteria for Dynamic photothermotherapy with Indocyanine Green (*i*-PTT) for the treatment of classics and/or occult subretinal neovascular membranes, and can also be understood for other types of neovascular membrane mixed, classics, occults, predominantly classics, predominantly occults, exclusively classics, exclusively occults for several causes and different extensions and localizations and complications, associated or not with choroidal retinal, subretinal hemorrhage and/or concomitant cicatrizing processes. This invention is proposing a new use of Indocyanine Green that according to aforesaid report is a safety photosentive dye that can be easily administered with rapid distribution and excreted.

Up to now, there is no knowledge in specific literature on this issue, referring to the application of this substance, associated with a wavelength laser at 700 to 900 nm preferably 810 nm of low power inferior to 1.000 mW and with extended exposure time at 40/150 sg, preferably 100 sg, for beams of 1.600 up to 8.600 micros, thereby generating retinal irradiances never higher than 4 mW per cm², thereby, conferring a satisfactory response, primarily by means of photooxidative effects type II, with irrelevant heat generation as in photosensitizing techniques applied in dynamic photothermotherapy and selective dynamic

thermotherapy of ophthalmologic field with other photosensitizing agents, but specifically either in patent literature or in scientific publications, up to now, there is no knowledge of the potential of dynamic photothermotherapy and selective dynamic thermotherapy for choriocapillaris and/or choroidal neovascularization and subretinal using Indocyanine Green as photosensitizing agent, as from the evidence that the substance at issue shows characteristics of spectral penetration that facilitates the choroidal and choriocapillaris angiographic delineation, where the neovascular membrane originates, the creators of the present invention have associated this fact to its activation.

This fact was associated with its activation at 805 nm bands that warrants greater tissue penetration of the laser due to the wavelength band at 800nm.

Thus, using a light excitation at low power (retinal irradiance lower than 4,0 W/cm2), extended exposure time (60 and 150 seconds/ preferably 100 seconds), and with wavelength at 700 and 900nm, specifically 805nm, the dynamic photothermotherapeutic effect and selective vascular occlusion of the treated region, was reached in an efficient form.

The evidence that, further to the fluorescence properties, indocyanine green shows, as other second-generation agents used for photodynamic therapy, a low phototoxicity for the skin, selectivity for the targeted tissue, rapid delivery and biodistribution, easy administration and monitored made the inventors investigate the possibility of associating the application of referred substance in photosensitizing techniques (photodynamic therapy), aiming at achieving an efficient photosensitizer for dynamic photothermotherapy, to be used in ophthalmologic

field, in therapies for subretinal neovascular membranes, particularly, in age-related macular degeneration, pathological myopia, angiode strias, dystrophies, traumas and inflammations.

The present invention arises with great impact to reveal a new use for indocyanine green that is normally used in achieving the angiography image with indocyanine green, for recording retinal vasculature, mainly choroidal (vascular tissue situated right behind the retina and is responsible for nutrition of inner retinal layers). Thus aiming at occlusion or specific selective closing of neovascular membranes of patients with age-related macular degeneration, pathologic myopia, angiod strias of idiopathic and/or traumatic or inflammatory causes and/or related to dystrophies, primarily through photo-oxidation reactions type II, with generation of singlet oxygen through braking out a thrombosis cascade by disintegration of red blood cells, release of active oxygen, oxidative radicals, fibrin and circulation obstruction in choriocapillaris, reaching, thereby, an efficiency of 100% positive results (obstruction of targeted vessels) in present technique.

The Researchers of the present invention of dynamic photothermotherapy with indocyanine green have concluded that this method (*I*-PTT) allows endothelium-bound intraluminal photothrombosis, able to spare important structures for the vision such as the neurosensory and Brüch's membrane.

Water-solubility, fluorescent properties and activation at 805nm make indocyanine green an ideal photosensitizer for choroidal neovascularization. These combined considerations point towards the need of diverse and deep studies, among them, performance of clinical analysis,

clinical studies, double blind, randomized for the treatment of choroidal neovascular disease related to several causes.

Regarding the mechanisms of action of photosensitizer-induced cytotoxicity, indocyanine green, the photosensitizer molecule that has absorbed laser light energy can reach the ground state activated by radioactive or non-radioactive mechanism.

In non-radioactive mechanism, the molecule looses the energy absorbed by the molecule and can be converted to heat, by internal conversion or transferred to other molecules by means of a photo-oxidation reaction type I, thereby damaging cells by raising their intracellular temperature, created by the use of photocoagulation implemented by indocyanine green (Reichel E, Puliafito C, Duker J, Guyer D. Indocyanine green dye enhanced diode photocoagulation of poorly defined subfoveal choroidal neovascularization. *Ophthalmic Surg* 1994; 25:195-201) or tissue welding (Decoste S, Farinelli W, Flotte T, Anderson R. Dye-enhanced laser welding for skin closure. *Laser Surg Med* 1992; 12:25-32).

Alternatively, indocyanine green dye may release the energy absorbed by the photosensitizer, transfer it to the oxygen molecule by means of a photo-oxidation type II, via a triplet state, and other components to form reactive intermediates as singlet oxygen, which can cause irreversible destruction in biological substrates. Other reactive species such as superoxide, hydroperoxyl, or hydroxyl radicals may also be involved in the irreversible damage mediated by biological components (Henderson BW, Dougherty TJ. How does photodynamic therapy work? *Photochem Photobiol* 1992; 55:145-157, Roberts W, Hasan T. Role of neovasculature and vascular permeability on the

tumor retention of photodynamic agents. *Cancer Res* 1992; 52:924-930).

Singlet oxygen flows at a reactive distance of only 0.1 μm, so that cytotoxicity is restricted to the immediate vicinity of the photoactivated drug. At the doses of photosensitizer and light used, neither the light nor the sensitizer had any independent activity contrary to the targeted tissue. Occlusion of the vascular bed reached as major mechanism of action of photodynamic therapeutic process, arises after damage to endothelial cells with subsequent platelet adhesion and thrombus formation. Indirect evidences suggest that the primary photodynamic reaction is a type II photosensitization mediated by singlet oxygen.

Studies with photosensitizers have shown differences in singlet oxygen quantum: 0.29 for hematoporphyrin derivative; 0.36 for phthalocyanines; 0.67 for purpurins; 0.77 for NPe_6. The triplet oxygen quantum by indocyanine green is 0.14 in water and 0.16 in methanol, and 0.17 in dimethyl sulphoxide (Reindl S, Penzkofer A, Gong S-H, *et al.* Quantum yield of triplet formation for indocyanine green. *J Photochem Photobiol A* 1997; 105:65-68).

In comparison to other photosensitizers, the triplet energetic fields of Indocyanine Green appear low, but they were sufficiently high to induce photodynamic therapy in the present study. (Miller JW, Walsh AW, Kramer M, *et al.* Photodynamic therapy of experimental choroidal neovascularization using lipoprotein-delivered benzoporphyrin. *Arch Ophthalmol 1995;* 113:810-(818).

Studies performed by us have shown that indocyanine green is an efficient photosensitizing agent for normal choriocapillaris occlusion in pigmented or non-pigmented rabbits. The study of choriocapillaris with laser alone,

without the photosensitizer, using the same parameters as in dynamic photothermotherapy, in previous experiments performed, showed no alterations in choriocapillaris, choroids or in the retina, as shown by histology and/or fluorescein angiography, and further, this alteration was not clinically evident during and after the therapeutic process. Due to this fact, we can affirm that occurred no thermal lesion of the tissues treated, which were targeted by photodynamic therapy. Using indocyanine green, the subretinal vasculature was efficaciously closed, with minimal alteration in adjacent neurosensory retina with light doses, as low as 7,2 J/cm^2. More intense choroidal effects were noted at the highest doses of light tested. Clinical examination and fundus photography revealed no retinal color change during and immediately following the use of dynamic photothermotherapy treatment, although a mild grayishing of the neurosensory retina was noted on the next day. Additionally, fluorescein angiography applied to group V of the experiment showed choroidal occlusion evidently larger after 24 hours in comparison to the early fluorescein angiography performed 6 hours after light exposure. The delayed response is also a sign that the mechanism of vascular occlusion action was photochemical and not thermal. Although choriocapillaris occlusion was associated with a mild damage at level of retinal pigment epithelium in our study, this alteration also occurred in several other studies. The extent of the damage depended on the dose of light administered and was noted in the treatment field. Any extension in its vicinity, as noted in other types of treatment, caused by lateral, deep and superficial heat transference, which arises with thermal photocoagulation. (Wilson CA, Royster AJ, Tiedeman JS, Hatchell DL. Exudative

Retinal detachment after photodynamic injury. *Arch Ophthalmol* 1991; 109:125-134., Schmidt UE, Hasan T, Gragoudas E, *et al.* Vascular targeting in photodynamic occlusion of subretinal vessels. *Ophthalmology* 1994; 101:1953-1961.,Peyman GA, Moshfeghi DM, Moshfeghi A, *et al.* Photodynamic therapy for choriocapillaris using tin ethyl etiopurpurin (SnET2). *Ophthalmic Surg Lasers* 1997; 28:409-417.) at the lowest irradiation levels, even in the absence of choriocapillaris PDT injury (Yao X-Y, Marmor MF. Induction of serous retinal detachment in rabbit eyes by pigment epithelial and choriocapillary injury. *Arch Ophthalmol* 1992;110:541-546)

Additionally thermal photocoagulation induced by laser, leads to much more extensive destruction of pigment epithelium cells and of photoreceptors rather than photodynamic therapy due to the fact that pigment epithelium is the tissue able to absorb larger quantity of light energy in this method, whereas in the method that uses light photosensitization, the most damaged area is primarily confined to the targeted vascular tissue (Wilson CA, Royster AJ, Tiedeman JS, Hatchell DL. Exudative Retinal detachment after photodynamic injury. *Arch Ophthalmol* 1991;109:125-134., Schmidt UE, Hasan T, Gragoudas E, *et al.* Vascular targeting in photodynamic occlusion of subretinal vessels. *Ophthalmology* 1994;101:1953-1961.,Peyman GA, Moshfeghi DM, Moshfeghi A, *et al.* Photodynamic therapy for choriocapillaris using tin ethyl etiopurpurin (SnET2). *Ophthalmic Surg Lasers* 1997;28:409-417) at the lowest irradiation levels, even in the absence of choriocapillaris PDT injury (Yao X-Y, Marmor MF. Induction of serous retinal detachment in rabbit eyes by pigment epithelial and choriocapillary injury. *Arch Ophthalmol* 1992;110:541-546)

Indocyanine green is not significantly toxic in animals, since its lethal dose (LD50) after intravenous administration ranges from 50 to 80 mg/kg. There are no equivalent data in humans; the disappearance rate of indocyanine green in healthy human subjects is 18 to 24% per minute. The normal biologic half-life was determined finding the value of 2.5 to 3 minutes so that blood samples taken from healthy patients 20 minutes after the injection of indocyanine green does not contain more than 4% of the initial concentration of the dye (Villeneuve JP, Huot R, Marleau D, Huet PM. Estimation of hepatic blood flow with indocyanine green: Comparison between the continuous infusion and single injections methods. *Am J Gastroenterol* 1982; 77:233-237) eliminating the need for a 24-hour period of light abstinence as required for photodynamic therapy with verteporfin.

Indocyanine green is not metabolized after an intravenous injection, and is excreted exclusively through hepatic via. It is not absorbed from the intestine and does not stay in enterohepatic circulation. The dye is taken from the plasma by the hepatic parenchyma cells and secreted into the bile. Negligible uptake of the dye occurs in the kidneys, lungs, cerebrospinal fluid, and peripheral circulation. Renal excretion does not occur. The safety of intravenous administration of indocyanine green in humans was already well documented, with severe adverse reactions occurring in only 0.05% of patients. Although greater concentration levels of the second-generation agents for photodynamic therapy with benzoporphyrins, purpurins, phthalocyanines, mono-L-aspartyl-chlorine-6 were determined in experimental studies, the distribution and retention of these drugs in human neovascular tissue is currently

extrapolated from pretreatment conditions, reached by means of fluorescein angiography and the concentration of sodium fluorescein in neovascular membrane rather than concentration of photosensitive substance in neovascular membrane. These data may not properly reflect the biodistribution of the photosensitizer in the eye and its association with the targeted field to be treated, which does not occur with indocyanine green that has the angiographic method as investigation of its concentration prior to the indication of the treatment itself, thus, the uptake specificity of the neovascular membrane for this photosensitive dye can already be noted, prior to the proper treatment indication. Taking a particular patient as basis, it is theoretically possible to associate the properties of fundus imaging concomitant with the phototherapeutic properties of indocyanine green to optimize either the propedeutic or disease treatment in only one section. Photodynamic therapy with existing ophthalmic agents is mainly limited by poor aqueous solubility, needing intralipid or liposome formulation and consequent intravenous administration by slow infusion, in contrast with the application of indocyanine green that is a water-soluble substance and can be rapidly infused, thereby avoiding long intervals of 10 minutes or more, required for slow infusion of a preparation for suspension with lissome and/or with intralipid formulation. This property also enhances treatment practicality and avoids the fact of increasing patient compliance and comfort, in addition, modification of indocyanine green with encapsulation can and other chemical modification can also be rapidly administered, thereby enhancing the photothermodynamic effect.

Currently, in addition to having a high molecular weight 775 kDa, indocyanine green is bound rapidly and almost

completely (98%) to plasma proteins. Albumin is its major carrier; however, in human serum, 80% of indocyanine green can be bound to globulins, probably alpha-1 lipoproteins (Baker KJ. Binding of sulphobromophtalein (BSP) sodium and indocyanine green (ICG) by plasma alpha-1 lipoproteins. *Proc Soc Exp Biol Med* 1966; 122:957-963) that have hindered a vascular retention and minimal leakage from abnormal vessels (Ham W, Sliney D. Retinal sensitivity to damage from short wavelength light. *Nature* 1976; 260:153-155) Like choroidal neovascularization, tumor neovasculature is highly proliferative. (Denekamp J. Vascular attack as a therapeutic strategy for cancer. *Cancer Metastasis Rev* 1990; 9:267-282).

The hyperproliferative neovascular tissue exhibits an enhanced permeability and elevated levels of specific albumin and LDL receptors (Schmidt U, Birngruber R, Hasan T. Selective occlusion of ocular neovascularization by photodynamic therapy. *Ophthalmology* 1992; 89:391-394) as well as LDL receptors (Rutledge J, Curry F, Blanche P, Krauss R. Solvent drag of LDL across mammalian endothelial barriers with increased permeability. *Am J Physiol* 1995; 268:H1982-H19910 leading to increased LDL transport across endothelial junctions (Schnitzer J, Carley W, Palade G. Albumin interacts with a 60-kDa microvascular endothelial glycoprotein. *Proc Natl Acad Sci* 1988;85:6773-6777).

In order that a dye can have a photodynamic action, the photosensitizer must be irradiated by a non-thermal low intensity light, and at the wavelength of greater absorption for the photosensitizer.

Indocyanine green reveals a high absorption in the infrared spectrum region around 805 nm. Infrared light penetrates deeper into tissue of red light, thereby

conferring advantages in the selectivity for treatment of subretinal or choroidal neovascular membranes.

This study reveals the ability of indocyanine green to efficiently close the choriocapillaris or choroidal neovascularization while sparing the neurosensory retina. Even at doses as high as 20 mg/kg, like those used in experiments with pigmented and non-pigmented rabbits, histopathologic examinations revealed preservation of the neurosensory retinal architecture, with minimal loss of visual cells. Damages were only noted in choriocapillaris endothelial cells and in pigmented epithelium cells.

The increase of indocyanine green dose from 10 mg/kg to 20 mg/kg at an appropriate light fluency, choriocapillaris occlusion and the breakdown of blood-retinal barrier in rabbits became more evident after fluorescein angiography within 6 to 24 hours post-experiment. Lesions considered optimal with the minimum damage in neurosensory retinal tissue could be achieved with both doses with a light flux as low as 7.2 J/cm^2. Thus, indocyanine green in its regular and/or single conventional form and in presentations with modifications is a safety photosensitizer that can be widely used in ophthalmology with very low local or systemic toxicity effect and is easily administered and is rapidly distributed and excreted. Its protein binding characteristics and its chemical structure favor its localization and retention in abnormal vessels, rather than normal vessels. Furthermore, its spectral penetration characteristics facilitate the angiography with prior delineation of the choroidal tissue and/or choriocapillaris and/or neovascularization for the subsequent treatment. Additionally, activation at 805 nm allows a deeper light penetration. Considering all facts mentioned, these facts

indicate that further and deeper diverse studies with the proposed dynamic photothermotherapy process, the i-PTT, the standardization of subretinal neovascular membrane are desire and necessary for the expansion of the method.

The present invention reveals a new use of indocyanine green, in any form of presentation, as an efficient photosensitizer substance for use in the dynamic photothermo therapy and in the selective dynamic thermotherapy for selective occlusion of subretinal neovascular membranes, mainly by means of photooxidation reactions type II. The referred substance is applied in photosensitizing compound form.

Indocyanine green, in its photosensitizing form, was reconstituted into solution form, using 0.5 to 10 ml, preferably 2 ml of sterile distilled water for 50 mg of indocyanine green in its powder form. The solution reached was maintained and protected from light during the handling of the indocyanine green to prevent inadvertent activation.

The objective of the present invention refers to the use in treatment of subretinal neovascular membranes, through photooxidation reactions type II (photodynamic therapy) reached with the use of indocyanine green and a laser delivery system with modified diode and slit-lamp. The diode laser has the wavelength of 810 nm in infrared spectrum band, near the maximum absorption peak of indocyanine green and has a diaphragm mechanism with different openings for exposure of beams with different diameters 0.8, 1.0, 1.2, 1.5, 2.5 and 4.3 mm, coupled to the co-observation system with biomicroscope in slit-lamp form.

In a modality of use of the present invention it is foreseen for the indocyanine green as the photosensitizing agent in the treatment of dynamic photothermotherapy and of

selective dynamic thermotherapy for occlusion of neovascularization of the coróide, its presentation in packages from 100 to 200mg, in one or two flasks, being used in therapeutic dosage from 1 to 5 mg/kg per weigth, more preferable from 2 to 3mg/Kg, being the package linked during the treatment above refered, in flasks containing from 125 to 150 of the powder of the dye.

That is due to the fact that the dye can be administered in three ways in relation to the time of application of the laser: - immediately before, 30 minutes before and another combined (30 minutes before and immediately before), in that to occur the accumulation of the dye to obtain higher effect, the two applications overlaying itself. The filling of photosensitizing agent could be made by the packaging of the volume foreseen in one or in two flasks.

For illustrative purposes the following figures are presented, and above procedures can be noted for a better understanding of the present invention that has the essential photodynamic effect as basis, with a laser delivery system at 810 nm with beams of large diameters (over 1500 micros in the retina).

FIGURE 1:

Group 5, control eye. 20 mg/kg of indocyanine green, 130 mW.

(Top) Aneritra Red-free fundus photography, one day after the injection of distilled water, and light treatment at 810 nm, at doses of 1.3, 2.6, and 3.9 J/cm^2. No retinal discoloration and/or whitening were noted within the treatment field. Note marker burns just below the treatment field as points of reference for observation of the dynamic photothermotherapy fields (arrow).

Bottom: late-phase of the angiogram with sodium fluorescein in the same eye, no leakage or abnormal fluorescein was present, indicating that there was no thermally induced alteration or effect of the distilled water alone in pigment epithelium or alteration in blood-retinal barrier.

FIGURE 2:

Aneritra Red-free fundus photography and fluorescein angiography 24 hours post *i*-PTT. (Top) Group 1 - 10 mg/kg of indocyanine green, 50 mW: no significant retinal alterations were noted (Middle). Group 3 - 10 mg/kg of indocyanine green, 90 mW: moderate retinal discoloration or grayishing was noted with 21.6 J/cm^2 in the *i*-PTT treatment field (arrow). Late-phase of the angiogram with fluorescein in the same eye showed hyperfluorescence in the three fields treated with *i*-PTT, and a more evident fluorescein leakage was noted in the center of the field treated by 21.6 J/cm^2. (Bottom) Group 4 - 20 mg/kg of indocyanine green, 90 mW: moderate deep retinal discoloration was noted in the fields treated with *i*-PTT. Late-phase of the angiogram with fluorescein in the same eye showed areas of hyperfluorescence in the three fields treated with *i*-PTT, and a more intense fluorescein leakage was noted in the center of the field treated by 14.4 and 21.6 J/cm^2.

FIGURE 3:

Group 5 - eye treated with *i*-PTT of group 5 (20 mg/kg of indocyanine, 130 mW). (Top) Small hypofluorescent lesions were noted in the center of the treatment fields, in the early-phase of fluorescein angiography, six hours after light exposure (arrows). (Bottom) Fluorescein angiography

at 24 hours after the treatment, showing evident choroidal occlusion increased in the fields treated with *i*-PTT (arrows), and intense leakage was noted in the late-phases of angiogram on the same eye.

FIGURE 4:

Light micrograph nine days after light treatment. (Top) control eye of group 5 (distilled water), 130 mW). Areas of retina and choroids, including photoreceptors, appear unaltered after irradiation at 30,0 J/cm^2 (toluidine blue; original magnification x200, bar: 20 µm). (Bottom) group 1 (10 mg/kg of indocianine, 50 mW). After irradiation, no alteration in the retina or choroids was noted (toluidine blue; original magnification x500, bar: 10 µm).

FIGURE 5:

Light photography and electronic transmission electron micrograph, after 9 days post light treatment. (Top) Group 3: 10mg/kg of indocyanine, 90 mW/cm^2). Choriocapillaris occlusion was found after an irradiation dose of 14.4 J/cm^2. In contrast, the overlying neurosensory retina was well preserved, exhibiting only a minor loss of photoreceptor external segments. No occlusion of large and deep choroidal vessels was found (toluidine blue; original magnification x200, bar: 30 µm). (Middle) (Group 4) 20 mg/kg of indocyanine green, 90 mW. Alterations in the choriocapillaris cytoplasm endothelial cells characterized by vacuolization and electron dense inclusions. The melanosomes were intact, excluding any thermal mechanism or damage caused by photocoagulation induced by laser (uranyl acetate; original magnification x5000, bar: 2 µm). (Bottom) (Group 4) (20 mg/kg of indocyanine green, 90 mW). Although eventual

fibroblasts infiltration and disappearance of the basal infoldings were found, Brüch's membrane was intact. The disorder of pigment epithelium cells were also noted (uranyl acetate; original magnification x5000, bar: 1.8 µm)

FIGURE 6:

Light micrograph and electronic transmission electron micrograph, nine days after light treatment. (Top left) Lesion of 21.4 J/cm^2 of group 3 (10 mg/kg of indocyanine green, 90 mW). Choriocapillaris was occluded with deeper choroidal vessels remaining perfused. The outer nuclear layer was intact, with no damage to inner retinal layers (toluidine blue; original magnification x200, bar: 10 µm). (Top right) Lesion of 14.2 J/cm^2 of group 4 (20 mg/kg of indocyanine green, 90 mW). An erythrocyte and fibrin were filling the lumen of a choriocapillaris. The pigment epithelium cells were disrupted (uranyl acetate; original magnification x8000, bar: 1 µm). (Bottom left and right) Lesion of 14.2 J/cm^2 of group 4 (20 mg/kg of indocyanine green, 90 mW). Choriocapillaris endothelial cells showed cytoplasm projections, folding and infoldings further to vacuolization. Bruch's membrane appeared normal. Some endothelial cells of the choriocapillaris revealed loss of intercellular junctions (arrows) (uranyl acetate; original magnification x12k/x40k, bar: 500/100 nm).

FIGURE 7:

(Top left) Light micrograph two days after light treatment of a 3.9 J/cm^2 lesion of group 5 (20 mg/kg of indocyanine green, 130 mW). Eventual occlusion of choroidal deep vessels associated with complete choriocapillaris occlusion was noted without hemorrhage. The photoreceptor layers showed vacuolization and edema. The inner retina

layers appeared unaltered (toluidine blue; original magnification x200, bar: 18 μm). (Top right) Transmission electron micrograph in the same lesion showing the choriocapillaris filled with a combination of erythrocytes cells, neutrophilis, platelets and fibrin. The melanosomes were intact, and having some degree of lesion in pigment epithelium cells. Brüch's membrane remained intact, but there was damage to the outer retina layer, and with photoreceptors forming rings (uranyl acetate; original magnification x3000, bar: 2 μm). (Bottom left) The photoreceptor segments in higher magnification showed degeneration characterized by fusion of disc membranes with convoluted aspect, sometimes associated with the disruption of internal segment (uranyl acetate; original magnification x8000, bar: 1 μm). (Bottom right) Light micrograph taken 9 days after light treatment of a 30.0 J/cm^2 lesion of group 5 (20 mg/kg of indocyanine green, 130 mW). Although the choriocapillaris remained occluded, the deep and largest choroidal vessels were perfused, in contrast to the retinal lesion noted; after 2 days of light exposure, the outer retina was severely damaged probably due to prolonged ischemia. The pigment epithelium and photoreceptor cells were fully disordered and disrupted. The outer nuclear layers showed piknosis and vacuolization (toluidine blue; original magnification x200, bar: 20 μm).

Illustrating Examples:

Example I:

Photosensitizing compound indocyanine green (ICG-Pulsion, Munich) was reached in dehydrated powder form, 50mg. The photosensitizer dye indocyanine green was reconstituted into solution form using 2,0ml of distilled water at 5°C during one hour, reaching a reconstitution in aqueous solution form. The solution was maintained protected from

light and immediately reconstituted prior to its use and protected from light during all handling period, in order to prevent inadvertent activation.

Example II:

The laser delivery system consisted of a laser in infrared diode spectrum and a biomicroscope in slit-lamp form. A diode laser (TTT 1500, OPTO, São Carlos, Brazil) with wavelength at 810 nm, near the maximum absorption peak of indocyanine green. The application beams consisted of diaphragms with 0.8, 1.0, 1.2, 1.5, 2.5, and 4.3 mm diameters.

Example III: Animal models

All animals were treated in accordance with the resolution of the ophthalmology association for research in vision on the use of animals in research. Fifteen pigmented rabbits weighing from 2 and 2½kg were examined by indirect ophthalmoscopy and considered to have normal fundi, already at the beginning of the study. Anesthesia consisted of an intramuscular injection of ketamine hydrochloride (50 mg/kg) plus xylazine hydrochloride (5 mg/kg) during all procedures, with additional ketamine injections as needed. Pupillary dilation was reached using topical tropicamide 1% and phenylephrine hydrochloride 2,5%. Eyes were enucleated under deep anesthesia, and the animals were euthanatized with an intravenous injection of pentobarbital sodium (50 mg/kg).

Example IV: Dynamic Photothermotherapy

Prior to light application, a fundus contact lens (Mainster, Wide Field, Ocular Instruments, Belleviwe, USA) was placed on the rabbit's cornea. In order to facilitate tissue sectioning for histological examination, three intense marker burns were produced in both eyes by photocoagulation

in the inferior paramedullary region. Three areas on the left eye were exposed to the following light duration schedule (temporal to nasal): 20, 30, and 10 seconds. The diameter of each light stimulating shot in the retina was equal to 4000 μm. The intensity used was 50 mW (Group 1 and 2), 90 mW (Group 3 and 4), or 130 mW (Group 5). The power densities chosen for this study were based on the results of preliminary work in the same rabbit model, indicating the amount of light necessary to induce thermal damage. (Costa et al, unpublished data, 2000). These parameters resulted in total fluencies of 4.2 to 30.0 J/cm². The rabbits had their marginal ear vein used for intravenous bolus injection, either 10 mg/kg (Group 1 and 3) or 20 mg/kg (Group 2, 4 and 5) of indocyanine green followed by a saline flush.

Control group consisted of fellow eyes that were treated in the same manner after injection of an equivalent volume of sterile distilled water. Light irradiation started 10 seconds following the intravenous injection of either indocyanine green or distilled water.

After treatment, the animals were returned to their housing and raised under ordinary fluorescent lights, 30 lux for 9 days. Fundus photography and fluorescein angiography were performed 24 hours after treatments, using a fundus camera (TRC-50IA/IMAGEnet, Topcon, Tokyo, Japan). Additional photography and angiography were performed 6 hours after light treatments in the group 5. The photography was performed with 10% sodium fluorescein (0.1 ml/kg) via marginal ear vein injection.

Example V: Histological analysis

By the ninth day, the eyes were enucleated and the rabbits were euthanatized. Additional analyses were also

performed two days after light exposure in one animal of group 5. Immediately after enucleating, the eye was incised at the equator and the vitreous was removed. All specimens were fixed by immersion in 2.5% glutaraldehyde in 0.1M cacodylate buffer (pH 7.4) for two hours at 25° C. On the next day, the tissue was then transferred to 0.1 M cacodylate buffer. The lesion was individually dissected and post-fixed in 2% osmium tetroxide in 0,1 M cacodylate buffer for one hour at 25°C. After several rinses in water the specimen was stained overnight in 0.5% (W/V) aqueous uranyl acetate. The specimen was dehydrated in equal series of alcoholic solution, followed by propylene oxide. The tissue was imbibed in epoxy resin and sectioned at 0.5 μm.

Example VI:

Findings in Fundus Photography and in Fluorescein Angiography, ninety treatment areas, in total, were irradiated in this study. There was no color change in any treated area during or immediately after the region injured by light stimulation.

In control eyes of the five group animals that were injected with distilled water and exposed to light doses, according to the methodology, revealed no fundus photography or fluorescein angiography alteration in the treated areas, indicating that there was no thermally induced alteration or other effect caused by aqueous solution, only on the pigment epithelium or on the integrity of the blood-retinal barrier (Figure 1).

Due to the fact that water is chemically inert, no photochemical reaction was noted in the fifteen control eyes.

No retinal alteration was shown within 24 hours after application of the dye or the light in groups 1 and 2 (50 mW).

Despite the fact that no color alteration was noted in the treated area, during or immediately following irradiation, 24 hours after treatment in the group 3 (90 mW), some degree of retinal discoloration or grayishing was observed in the treated areas that had received a light dose at 2.7 J/cm^2 starting from 24 hours.

In group 4 (90 mW) and group 5 (130 mW), all three fields treated by light stimulation showed retinal discoloration with well-defined limits. The degree of discoloration was dependent on the light dose administered.

Retinal edema was not present. There was no retinal or subretinal hemorrhage present.

In early-phases of fluorescein angiography there was a mild hypofluorescent lesion in the treated area in groups 3, 4 and 5.

The late-phases of angiogram showed hyperfluorescence indicating the breakdown of the outer blood-retinal barrier (Figure 2).

Six hours after light exposure, a mild hypofluorescence in the lesions in early-phases of the angiogram can already be seen in the centers of the treatment areas in the group 5.

Interestingly, fluorescein angiography at 24 hours showed evident choroidal occlusion enlargement (Figure 3).

Choriocapillaris occlusion and outer BRB breakdown were shown 24 hours post-treatment by fluorescein angiography at both tested dye doses and at a total light dose as low as 7,2 J/cm^2. No adverse systemic reaction to the dye was noted

Example VII: Histopathologic Alterations

Specimens from the ninth day post-treatment were obtained from two left eyes of each group and from one right eye of the respective control groups. The control eyes that had received laser and distilled water, as well as the lesions in groups 1 and 2, showed no evident alterations in retinal and choroidal architecture (Figure 4).

Lesions of the group 3 (7.2 and 14.4 J/cm^2) and group 4 (7.2 J/cm^2) revealed choriocapillaris occlusion and alteration of endothelial cells cytoplasm, characterized by vacuolization and electron dense inclusions. No occlusion of deep and large choroidal vessels was observed after nine days.

The Brüch's membrane was intact, although eventually existing fibroblast infiltration and disappearance of the basal folding and infoldings.

Disorder of pigment epithelium cells was observed, with melanosomes remaining intact.

Neurosensory retina was well preserved, exhibiting loss of some photoreceptor external segments only (Figure 5).

Lesions of the group 3 (21.6 J/cm^2), group 4 (14.2 J/cm^2), and group 5 (1.3 J/cm^2), revealed similar alterations in histological examination.

Choriocapillaris was filled with a combination of red blood cells and fibrin, whereas choroidal vessels remained open and perfused.

Endothelium of choriocapillaris cells showed cytoplasm projections, infoldings and vacuolization.

Some choriocapillaris endothelial cells exhibited loss of intercellular junctions.

Brüch's membrane remained unaltered and normal.

Pigment epithelium cells were disrupted and the outer nuclear layer was intact, with no evident lesion to inner retinal layers (Figure 6).

Choriocapillaris occlusion was also achieved in lesions of the group 4 (21.6 J/cm^2) and group 5 (20.0 and 30.0 J/cm^2). The choriocapillaris was filled with a combination of erythrocytes, neutrophilis, platelets and fibrin.

Pigmented epithelium cells were fully disrupted in some treated areas.

There was no damage to the outer retinal layers and the outer nuclear layer showed piknosis and vacuolization. The inner retinal layers were normal. No occlusion of deep and large choroidal vessels was found after 9 days.

There were significant differences in cytological aspects, when the analysis was performed two days after light exposure in lesions of the group 5.

Complete choriocapillaris and eventual deep and large choroidal vessels occlusion were noted. Brüch's membrane was intact. Mild alterations of pigment epithelium cells were noted, with some melanosomes remaining intact. The photoreceptor segments were disrupted resembling the ring aspect and some areas of apical condensation of pigment epithelium cells were noted.

Outer nuclear layers were edematous, and inner retina was normal.

In contrast with mild retinal alterations and pigment epithelium noted in treated areas after two days, the outer retina was severely damaged, with pigment epithelium cells fully disrupted after nine days, probably due to prolonged choroidal ischemia (Figure 7).

Example VIII:

A case of occult subfoveal choroidal neovascularization due to age-related macular degeneration was successfully treated with dynamic photothermotherapy, using indocyanine green and light irradiation with a diode laser at 810nm (*i*-PTT).

An 82-year-old patient developed loss of central vision in the left eye, prior to its presentation.

The best visual acuity reached with Snellen scale was RE: 20/25 and LE: 20/400.

A few druses were observed in the RE macula.

By fundus photography examination a subretinal neovascular membrane was observed centrally sub-fovea, beneath a serosanguineous detachment of pigment epithelial in the LE.

After the statement of procedure was informed and consented, the patient underwent a treatment with a modified diode laser with 810nm length (TTT 1500, Opto, São Carlos, Brazil) with a microscope with slit-lamp laser delivery system.

The diameter of the area to be treated was determined after digital measurement of the largest linear dimension of neovascular membrane, taking in consideration the sodium fluorescein angiography and indocyanine green, and 750 µm was added to this dimension to provide additional treatment around the lesion. A small volume of indocyanine green solution (1.5mg/kg) was then infused intravenously as a bolus, followed immediately by a saline solution flush. Two minutes after the flush infusion, 3.0 W/cm^2 laser light was applied to the lesion, during 95 seconds, through a contact lens for fundus biomicroscopy with 1.5X magnification power. The light application caused no alterations, visible by biomicroscopy or ophthalmology, concomitantly monitored to

the treatment, and soon after performance of the same. Color photographs, taken soon after performance of the treatment, indicating that there was no thermally induced lesion, confirmed this fact.

After treatment, visual acuity had improved to 20/200 and a hypofluorescent area corresponding to the serosanguineous pigment epithelium detachment could be identified by fluorescein angiography.

Retinal vessels remained perfused and showed no signs of edema and/or occlusion, even in the peri-foveal circulation.

Two months after treatment, visual acuity had improved to 20/160 and in ophthalmic and biomicroscopic examination there was a grayish ring corresponding to the initial lesion identified by indocyanine green angiography.

A complete regression of the subretinal neovascular membrane was achieved within one week post-treatment.

Visual acuity improved from 20/400 to 20/160 within two months of follow-up.

Optical coherence tomography revealed diminishing of the subretinal fluid. There were no complications related to the procedure.

CLAIMS

1. New use of indocyanine green characterized by the fact of being applied as a photosensitive agent in dynamic photothermotherapy and selective dynamic thermotherapy for choroidal neovascularization occlusion, Indocyanine at a dose of 10 to 20 mg/Kg and radiation as low as 0,9 Joules/cm^2.

2. New use in accordance with claim 1, characterized by the fact of being applied in occult choroidal neovascularization due to the Age Related Macular Degeneration.

3. New use in accordance with claim 1, characterized by the fact of being applied in the treatment of abnormal vessels of ocular tissues, particularly, in the choroids, in the retina and/or between the choroids and the retina, wherein fluid and/or blood are leaking to the referred tissues and thereby increasing the risk and/or causing visual decay.

4. New use in accordance with claim 1, characterized by the fact of being applied in its natural form and/or modified by liposome encapsulation and other chemical alterations, in a great photophysics and photochemical reaction affinity with an infrared laser duration that has great interaction with regular indocyanine green and/or modified by liposome encapsulation and other chemical alterations.

5. New use in accordance with claim 1,characterized by the fact of being used as a photosensitizing agent activated by a laser light excitation at low power, insufficient to generate a heat or photocoagulating hyperthermy and/or able of inducing tissue burns

6. New use of a laser therapy system and wavelength delivery in the infrared spectrum, characterized by the fact of aiming at achieving a photodynamic and selective vascular occlusion of the choroids and/or the retina or the subretinal space associated with indocyanine green defined in accordance with claim 1.

7. New use in accordance claim 6, characterized by the fact of the infrared laser being in a wavelength band that has greater affinity or absorption with photosensitizing indocyanine green.

8. New use in accordance with claim 6, characterized by the fact of achieving the intravascular clotting by photothermo-dynamic effect without significant damage, and/or tissues by heating, and/or by photocoagulating hyperperemy the adjacent tissues by the associated action of the dye and photosensitizing agent indocyanine green as an agent in photodynamic therapy.

9. New use in accordance with claim 6, characterized for being applied in the treatment of the diseases as age-related macular degeneration, pathologic myopia, angiod strias, presumed ocular histoplasmosis syndrome, inflammation or idiopathic causes and other abnormalities that may generate enlargement of abnormal vessels in ocular tissues.

10. New use in accordance with claim 6, characterized by the fact of occurring a photochemical and photophysics interaction with infrared wavelength light emitted by a diode laser to the exposure area of up to 8.6 mm diameter.

11. New use in accordance with claim 6, characterized by the fact of achieving an evaluation of the vascular tissues response, particularly, of the choriocapillaris and of choroidal neovascularization, in relation to indocyanine green, in relation/interaction to photophysics and

photochemistry (photodynamic) of indocyanine green and the light at a length of 810 nanometers.

12. Dynamic Photothermotherapy and Selective Dynamic Thermotherapy for subretinal vascular occlusion using infrared laser delivery system and indocyanine green characterized by the fact of involving:

(a) an intravenous injection of Indocyanine Green at doses from 0.5 to 5.0mg/Kg, per weight, preferably 2 mg/Kg,

(b) focal activation with laser light of 810nm in a retinal irradiance band where the energy absorbed by the molecule is not primarily converted into heat (photooxidation reaction type I), and thereby transferred to the molecular oxygen molecular via triplet state (photooxidation reaction type II).

13. Process in accordance with claim 12, characterized by the fact of the laser association in wavelength at 700 to 1.000 nanometers (preferably 810 nm), at lower power inferior to 1.000 miliwatts and prolonged time/disposition between 40 and 150 seconds (preferably 100 seconds), for areas circular applications of minimal 1.600 micros, thereby generate retinal irradiances never greater than 4.0 WATTS/square centimeter.

14. Process in accordance with claim 13, characterized by the fact of conferring a satisfactory response, primarily, by means of photooxidative effects type II, with stabilization or improvement of the acuity, in general, with improvement of the relative escotome, without collateral effects and with low rates of retreatment of the subretinal neovascular membrane,

15. Process in accordance with claim 12, characterized by the fact that the choriocapillaris occlusion was achieved

with Indocyanine at a dose of 10 to 20mg/Kg and radiation as low as 0,9 Joules/cm².

16. Process in accordance with claim 12, characterized by the fact that the use of Indocyanine Green at a dose of 10mg/Kg, results in absence of damages to the neurosensory retina.

17. Process in accordance with claim 12, characterized by the fact that the use of a light excitation at low power (retinal irradiance lower than 4.0 W/cm2), prolonged exposure time (60 to 120 seconds/preferably 100 seconds) and with a wavelength between 650 and 900nm, specifically 805nm, results in an effective dynamic photothermo therapeutic in selective vascular occlusion of the treated area.

18. Process in accordance with claim 12, characterized by the fact that the application of Indocyanine, associated with a wavelength laser at 700 to 1.000nm, preferably 810 nm of low power inferior to 1.000mW, and with prolonged exposure time at 40/150 seconds, preferably 100 seconds, for beams of 1.600 up to 8.600 micros, generates retinal irradiances never higher than 4 mW per cm², thereby, conferring a satisfactory response, primarily, by means of photooxidative effects type II, with irrelevant heat generation in ophthalmologic field.

19. Process in accordance with claim 12, characterized by the fact that the Indocyanine Green as the photosensitizing agent is presented in package from 100 to 200mg, in one or two flasks, being used in therapeutic dosage from 1mg to 5mg/Kg per weight, preferably from 2mg to 3mg/Kg, being the package linked during the treatment in flasks containing from 125mg to 150mg of the powder of the dye.

20. Process, in accordance with claim 12 or 19, characterized by the fact that the administration of the indocyanine green can happen in relation to the time of

application of the laser, immediately before the application, 30 minutes before, or in a combined way, 30 minutes before and immediately before, the two applications overlaying itself.

FIGURE 1

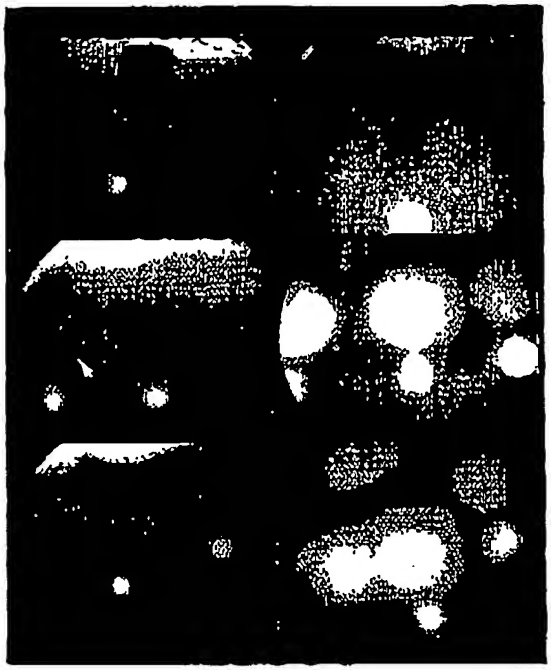

FIGURE 2

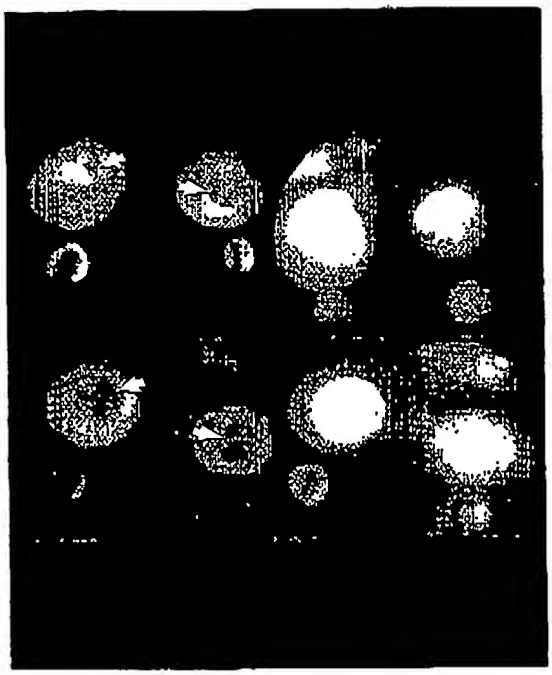

FIGURE 3

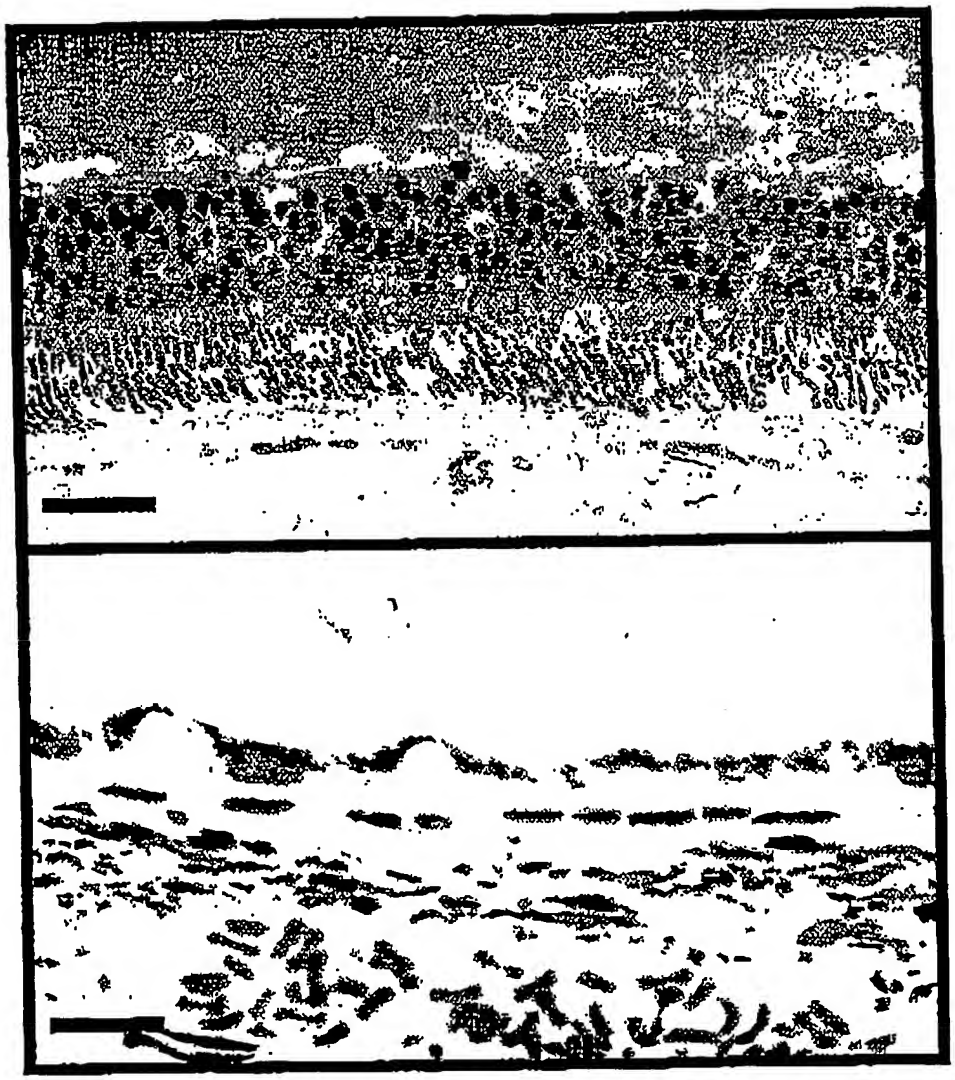

FIGURE 4

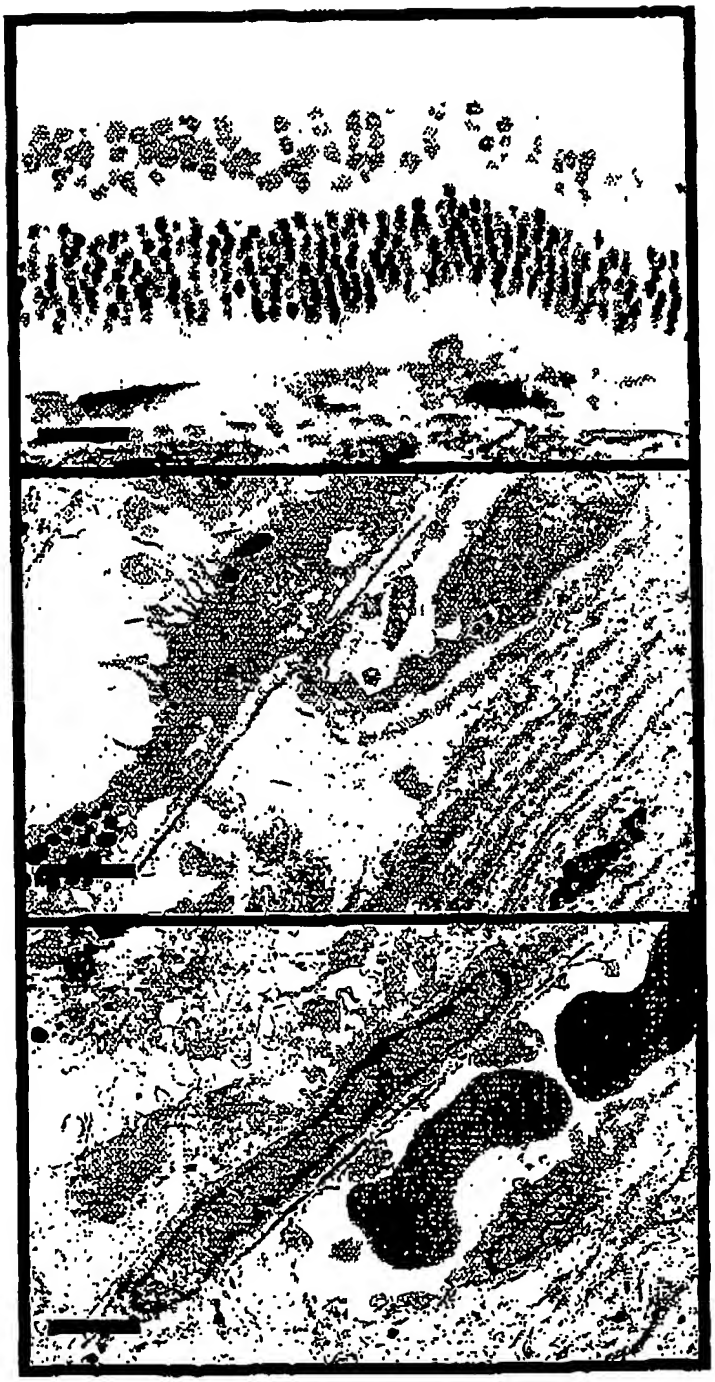

FIGURE 5

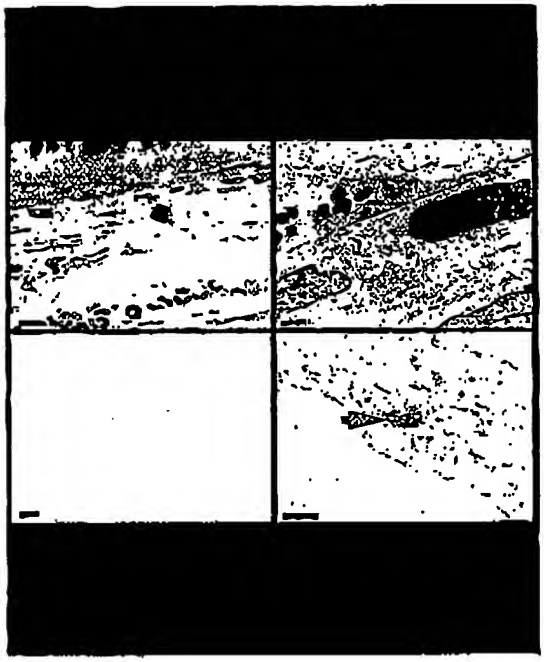

FIGURE 6

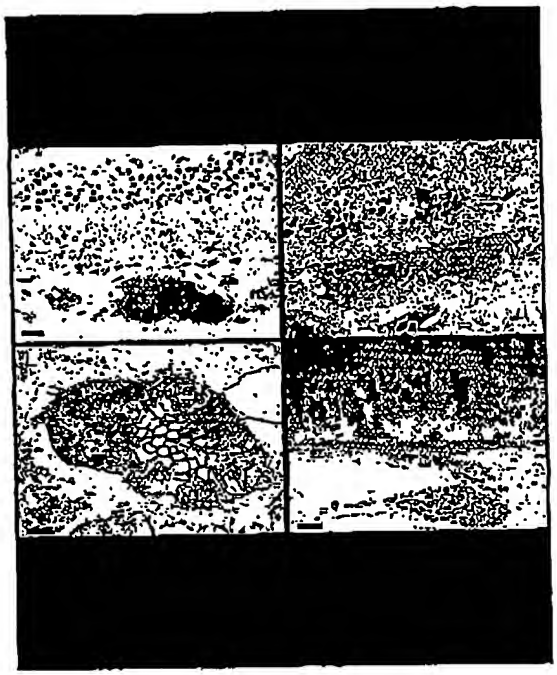

FIGURE 7

INTERNATIONAL SEARCH REPORT

International application No.
PCT/BR02/00010

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) :A61K 31/40
US CL :514/411
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 514/411

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 6,140,314 A (ZEIMER) 31 October 2000 (31.10.00), see the entire document.	1-20

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 30 MAY 2002	Date of mailing of the international search report 26 JUN 2002
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